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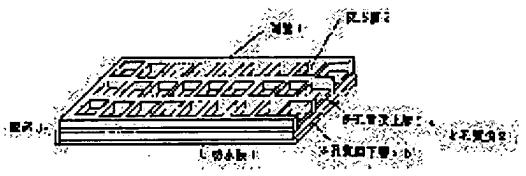
(54) GENE ANALYZER AND ANALYSIS

(57)Abstract:

PROBLEM TO BE SOLVED: To provide the subject apparatus capable of analyzing many genes at a time by installing a plural number of reactional vessels formed of a bottom formed of laminated plural number of porous membranes and partition walls formed in the upper part of the porous membranes.

SOLUTION: This gene analyzer is obtained by using a waterproof membrane 1 as the lowermost layer, installing a bottom 3 formed of a porous membrane 2 comprising at least two or more layers of a porous



membrane upper layer 2a and a porous membrane lower layer 2b different in material, using a material capable of functioning as a filter membrane and permeating genes and proteins without permeating cells as the membrane upper layer 2a and a material capable of functioning as a nucleic acid immobilizing membrane and immobilizing the genes on the layer without permeating the genes as the porous membrane lower layer 2b and then installing an assembly of a plural number of reactional vessels 5 formed of partition walls 4 in the upper part of the porous membrane 2. The resultant analyzer is capable of analyzing the many genes at a time by performing amplification and purification of the genes, immobilizing operations thereof onto the porous membrane and hybridization in the same reactional site.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the gene-analysis equipment and the approach the structure and the function of many genes are analyzable at once.
[0002]
[Description of the Prior Art] In recent years, the base sequences of the genome of many living things including the basis of remarkable progress of gene engineering and Homo sapiens are becoming clear, decode of all the base sequences of a yeast genome is completed at present, and it is expected that all future base sequences also with a near human genome will be decoded. However, a base sequence is decoded, and it cannot say that life information was decoded in the request, but it has been a very important technical problem in the life-science field to analyze each gene based on the information on the decoded base sequence.
[0003] Moreover, in the field of the clinical laboratory test, in case current detects the existence of virus infection, such as microorganisms, such as chlamydia, and HIV, HCV, before the symptoms of them is shown about illnesses of the gene origin, such as a hereditary disease and a gun, by conducting DNA inspection and investigating the gene in a genome in the future, it is predicted that performing a prediction diagnosis changes and increases to the present body fluid inspection.
[0004] In a gene analysis, electrophoresis of the strange gene which it is going to analyze, or the gene of known [array / a function and] is carried out on agarose gel. To porous membrane, such as a nitrocellulose and nylon film, (Southern-blotting Northern blotting) [whether imprint immobilization is carried out by the salt gradient, and] Or the solution containing a gene is dropped directly and it is made to fix on the film (dot blotting). The known gene which carried out the indicator with the radioactive substance after that, or a strange gene is supplied, hybridization is performed between the genes and marker genes which were fixed to the film, and detecting hybridization with autoradiography etc. is performed. However, the numbers of samples which can be processed when a gene is fixed by the blotting approach used now and hybridization is performed are 96 samples which used the at most 96 hole microtiter plate as the base, and are 5000-50000 at once like genome analysis. It has the problem that the number of samples of extent cannot be processed.
[0005] Moreover, although a gene may be supplied with the gestalt of a commercial reagent, after it is supplied by the host-vector system which used Escherichia coli etc. as the base and being amplified and refined by PCR, in many cases, analysis is usually presented with it. Usually, these actuation is performed by a micro tube and the microtiter plate, and it has the problem that many samples cannot be processed.
[0006]
[Problem(s) to be Solved by the Invention] The technical problem of this invention is by performing magnification and purification of a gene, fixed actuation to porous membrane, and hybridization all over a reaction-of-identity place to offer the gene-analysis equipment and the approach of processing many samples at once.

[0007]
[Means for Solving the Problem] The pars basilaris ossis occipitalis formed of two or more porous membrane by which the laminating was carried out as a result of repeating research wholeheartedly that this invention persons should solve the above-mentioned technical problem, The cell which contains a gene in the gene-analysis equipment which consists of two or more reaction vessels formed by the septum formed in the upper part of porous membrane is cultivated. By fixing a gene to porous membrane, performing hybridization between the this fixed gene and a gene with a complementarity, and detecting hybridization, after amplifying and refining a gene It finds out that the structure and the function of many genes are analyzable at once, and came to complete this invention.
[0008] That is, this invention is gene-analysis equipment which consists of two or more reaction vessels formed by the pars basilaris ossis occipitalis formed of two or more porous membrane by which the laminating was carried out, and the septum formed in the upper part of porous membrane.
[0009] The pars basilaris ossis occipitalis in which this invention is formed again of two or more porous membrane by which the laminating was carried out, In the reaction vessel of the gene-analysis equipment which consists of two or more reaction vessels formed by the septum formed in the upper part of porous membrane After inoculating the host cell holding the vector which has a gene, cultivating this cell and performing magnification and purification of a gene, It is the gene-analysis approach including fixing a gene to porous membrane, performing hybridization between the this fixed gene and a gene with a complementarity, and detecting hybridization.
[0010] This invention is the above-mentioned gene-analysis approach which reproduces the gene fixation film by making the cell in each reaction vessel adhere to the fixture for inoculation with which the projections of the shape of a rod positioned so that it might correspond to each reaction vessel gathered after the cell culture within a reaction vessel further, and transplanting to other equipments which installed new porous membrane.
[0011] After this invention inoculates the host cell holding the vector which has a gene, cultivates this cell and performs magnification and purification of a gene in the reaction vessel of the gene-analysis equipment which consists of two or more reaction vessels formed by the pars basilaris ossis occipitalis formed further again of two or more porous membrane by which the laminating was carried out, and the septum formed in the upper part of porous membrane, it is the manufacture approach of the gene-fixation film characterized by to fix a gene in porous membrane. Hereafter, this invention is explained in more detail.
[0012]
[Embodiment of the Invention]
[1] Gene-analysis equipment drawing 1 is the aggregate of two or more reaction vessels 5 formed by the pars basilaris ossis occipitalis 3 formed of the two-layer porous membrane (porous membrane upper 2a, porous membrane lower layer 2b) by which shows the gene-analysis equipment of this invention and the laminating was carried out to the waterproofing film 1 and it, and the septum 4 formed in the upper part of porous membrane.
[0013] the pars basilaris ossis occipitalis 3 of the gene-analysis equipment of this invention uses the waterproofing film 1 as the lowest layer, and an aperture differs from the quality of the material on it -- at least, the porous membrane 2 more than two-layer carries out a laminating, and is formed. Porous membrane usually consists of porous membrane upper 2a and porous membrane lower layer 2b. It will not be limited, especially if porous membrane upper 2a can function as filter film, and a cell cannot be penetrated as the quality of the material of porous membrane, but a gene and protein can be penetrated, and porous membrane lower layer 2b can function as nucleic-acid fixed film, and cannot penetrate a gene and a gene can be fixed on a layer. In order that DNA in a ****** reaction vessel may not mix through porous membrane, or the boundary part of the reaction vessels of porous membrane upper 2a fills up the hole of a porous body with a filler etc. and blockades, it is desirable to make a porous body dissolve partially with heat welding or a solvent, and to extinguish a hole. As the quality of the material of the waterproofing film 1, especially if a solution is not penetrated, it is not limited. An operating environment is humid, and the waterproofing film 1 has an enough DNA fixed capacity of porous

membrane lower layer 2b, when the desiccation from a pars basilaris ossis occipitalis can be prevented, and when DNA which was not adsorbed from a pars basilaris ossis occipitalis does not leak, it is not needed.

[0014] As the quality of the material of porous membrane, as for the upper layer, a glass filter, a cellulose filter paper, a cellulose acetate filter paper, a polycarbonate membrane, a cellulose acetate membrane, etc. are illustrated, and, specifically, as for a lower layer, a nitrocellulose membrane, a nylon membrane, a DEAE cellulose filter paper, a DEAE cellulose membrane, a PVDF membrane, etc. are illustrated. Specifically as the quality of the material of the waterproofing film, they are polyolefine films, such as polyethylene and polypropylene, and PET. High polymer films, such as a film and a polyvinyl chloride film, etc. are illustrated.

[0015] In the gene-analysis equipment of this invention, as for a reaction vessel 5, only the number of desired is formed in a desired configuration. The aggregate of a reaction vessel is beforehand formed in the shape of a sheet, and only the number of samples with which analysis is presented may be cut off and used.

[0016] considering as 0.001x0.001mm - 10x10mm (vertical x longitudinal direction) and a depth of about 0.001-10mm illustrates the size of a reaction vessel 5 -- having -- desirable -- 2x2 mm (vertical x longitudinal direction) and a depth of 0.1-10mm -- then, it is good. The volume of a reaction vessel 5 is 0.001ml - 0.2ml, although it can form from 1pl. It is desirable.

[0017] A septum 4 is formed in the upper part of porous membrane 2 so that each reaction vessel 5 may be divided and the component in an adjacent reaction vessel may not be mixed mutually. Although it will not be limited especially if it seems that DNA and protein are not adsorbed as the quality of the material of a septum, and the system of reaction is not checked, inorganic materials, such as polymeric materials, such as polyolefine and polyethylene, a metallic material, and silicon, are mentioned, for example.

[0018] As the formation approach of a septum, only a septum is formed beforehand and there is heat welding, adhesion, adhesion, an approach of installing on porous membrane with one means of the concavo-convex fitting by the cast, or the approach of forming by performing pattern spreading which has a certain amount of film pressure in porous membrane. Screen-stencil, roll coating, electrodeposition, nonelectrolytic plating, etc. can perform pattern spreading.

[0019] One or all of each class can carry out more independently than a septum desorption of the porous membrane which constitutes a pars basilaris ossis occipitalis. For that, along the part of a septum, pattern spreading of the binder is carried out, or concavo-convex fitting is installed in one or all of for example, porous membrane each class.

[0020] [2] After the gene-analysis approach of gene-analysis approach this invention inoculates host cells, such as a microorganism holding the vector which has a gene in the reaction vessel of the above-mentioned equipment, cultivates this cell and performs magnification and purification of a gene, it fixes a gene to porous membrane, performs hybridization between the fixed gene and a gene with a complementarity, and detects the existence of hybridization in a marker etc. As a marker, the radioactive substance, a fluorescent material, the fluorescence reagent to intercalate can be mentioned.

[0021] In the gene-analysis approach of this invention, all or a part of array and function set as the object of analysis may be strange genes (strange gene), or the gene fixed to porous membrane may be a gene (known gene) of array / functional known. Moreover, the fixed gene and the gene with a complementarity can be hybridized in the fixed gene, and a strange gene is used when the gene which fixes a known gene again when the gene to fix is a strange gene is a known gene. Hybridization between the genes and complementarity **** genes which were fixed is performed by detecting the indicator given to either of both the genes.

[0022] The supply to the reaction vessel of a host cell is supplied with a multiple-string dispenser robot or an inoculation fixture. Although a host cell and a vector system are properly used according to the class and the analysis purpose of a gene, the Escherichia coli and M13 phage which emits the vector of a single strand out of a fungus body especially in respect of purification processing are desirable.

[0023] Here, a gene can be amplified and refined by cultivating a host cell in a reaction vessel.

Moreover, in order to fix the gene concerned to a porous membrane lower layer, vacuum suction, electrical-potential-difference addition, centrifugal separation, etc. perform. The M13 phage which inserted the purpose gene in the porous membrane lower layer by this, leaving a fungus body to the porous membrane upper layer is fixed, and the gene fixation film is created. Washing processing, blocking, and heat treatment are possible for this film if needed.

[0024] Thus, the created gene fixation film is used for the gene amplification mentioned [which mentions later and hybridization-analyzes] later. Moreover, it can also be reproduced, being able to use the created gene fixation film as an original plate. First, the cell in each reaction vessel is made to adhere to the fixture for inoculation with which the projections of the shape of a rod positioned so that it might correspond to each reaction vessel gathered after the cell culture within a reaction vessel, and it transplants to other equipments which installed new porous membrane (plate which it is going to reproduce) (drawing 2).

[0025] Then, what is necessary is just to fix a gene to porous membrane, after cultivating this cell similarly and performing magnification and purification of a gene. Furthermore, the produced gene fixation film can be taken out, and it can put into the reaction vessel which put in the solution which contains a primer, dNTP, and Taq polymerase for that which reinstalled the septum, and can amplify by the PCR reaction (drawing 3). The primer used for PCR introduces the known array (consensus sequence for PCR) into the both ends of the gene inserted in a vector beforehand, and should just design it based on this array.

[0026] Although supply of the reaction mixture in each stroke, such as culture of a host cell, and growth, purification of a gene, is generally performed from the upper part, after removing the lowest layer (waterproofing film), it is possible through the porous membrane of a pars basilaris ossis occipitalis. Moreover, it is also possible by filling up a reaction vessel and porous membrane with an absorptivity material to gather the effectiveness of reaction mixture supply.

[0027]

[Example]

[Example 1] Drawing 4 shows one example of the gene-analysis equipment of this invention, uses the waterproofing film 1 as the lowest layer, and consists of two or more reaction vessels 5 formed by the pars basilaris ossis occipitalis 3 in which the two-layer porous membrane (2a, 2b) from which an aperture and the quality of the material differ carries out a laminating, and by which it is formed on it, and the septum 4 formed in the upper part of porous membrane.

[0028] M13 phage 8 (M13mp18) which included culture medium (culture medium for Escherichia coli culture) 6, the Escherichia coli fungus body 7 (Escherichia coli K-12), and various kinds of genes in each reaction vessel In addition, it is kept warm overnight [37 degree-C]. Within a reaction vessel, by culture of the Escherichia coli infected by M13 phage, phage increases, and a gene is amplified and refined.

[0029] After removing the waterproofing film 1 of the lowest layer after culture and carrying out multistory [of the absorptivity filter paper 9], it draws in from the lower part of the release side of another side to the pressurization or the absorptivity filter paper 9. Thereby, although culture medium shifts to the absorptivity filter paper 9 which carried out multistory, in that case, Escherichia coli remains on porous membrane upper 2a, and M13 phage is adsorbed on porous membrane lower layer 2b. Then, lower layer 2b is removed, fixed processing of M13 phage DNA is performed, and hybridization (complementation test) is performed by using as a probe gene the strangeness or the known gene which carried out the indicator by 32-P. The amount of indicator probes combined with M13 phage DNA on the film complementary is quantified using autoradiography and BAS1000 (Fuji).

[0030] [Example 2] Drawing 5 shows one example of the gene-analysis equipment of this invention, and consists of two or more reaction vessels 5 formed by the pars basilaris ossis occipitalis 3 in which the two-layer porous membrane (porous membrane upper 2a, porous membrane lower layer 2b) from which an aperture and the quality of the material differ carries out a laminating, and is formed, and the septum 4 formed in the upper part of porous membrane.

[0031] When the equipment concerned is installed for what carried out inoculation of the Escherichia

coli which infected each reaction vessel of this gene-analysis equipment by M13 phage, it cultivates by putting into the bat which put culture medium into the water level which does not exceed the height of a septum. Equipment is taken out after culture, and while attracting the culture medium which remained from the lower part with the vacuum aspirator, M13 phage is adsorbed and fixed at porous membrane lower layer 2b. Culture medium passes porous membrane lower layer 2b, and is attracted, and Escherichia coli is left behind to porous membrane upper 2a. Next, lower layer 2b is removed, fixed processing of M13 phage DNA is performed, and it is 32P. Hybridization (complementation test) is performed by using as a probe gene the strangeness or the known gene which carried out the indicator. They are autoradiography and BAS1000 (Fuji) about the amount of indicator probes combined with M13 phage DNA on the film complementary. It uses and quantifies.

[0032]

[Effect of the Invention] The gene-analysis equipment and the approach many genes are analyzable at once with this invention by performing magnification and purification of a gene, fixed actuation to porous membrane, and hybridization all over a reaction-of-identity place are offered. Moreover, the fixture for inoculation corresponding to the reaction vessel can reproduce the gene-analysis equipment of this invention by inoculating a cultured cell into other equipments.

[Translation done.]

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CLAIMS

[Claim(s)]
[Claim 1] Gene-analysis equipment which consists of two or more reaction vessels formed by the pars basilaris ossis occipitalis formed of two or more porous membrane by which the laminating was carried out, and the septum formed in the upper part of porous membrane.
[Claim 2] Gene-analysis equipment according to claim 1 with which the upper layer of porous membrane cannot penetrate a cell, but can penetrate a gene and protein.
[Claim 3] Gene-analysis equipment according to claim 1 which the lower layer of porous membrane cannot penetrate a gene, and can fix a gene on a layer.
[Claim 4] Equipment according to claim 1 in which the desorption of the porous membrane which constitutes a pars basilaris ossis occipitalis is more possible than a septum.
[Claim 5] Equipment according to claim 1 which has the waterproofing film in the lowest layer.
[Claim 6] In the reaction vessel of the gene-analysis equipment which consists of two or more reaction vessels formed by the pars basilaris ossis occipitalis formed of two or more porous membrane by which the laminating was carried out, and the septum formed in the upper part of porous membrane After inoculating the host cell holding the vector which has a gene, cultivating this cell and performing magnification and purification of a gene, The gene-analysis approach including fixing a gene to porous membrane, performing hybridization between the this fixed gene and a gene with a complementarity, and detecting hybridization.
[Claim 7] The gene-analysis approach according to claim 6 of performing PCR magnification after fixing a gene to porous membrane.
[Claim 8] The gene-analysis approach according to claim 6 which has arranged the known consensus sequence to the both ends of the gene inserted in each vector.
[Claim 9] The gene-analysis approach according to claim 6 which uses a host cell as Escherichia coli and makes a vector M13 phage.
[Claim 10] The gene-analysis approach according to claim 6 which reproduces the gene fixation film by making the cell in each reaction vessel adhere to the fixture for inoculation with which the projections of the shape of a rod positioned so that it might correspond to each reaction vessel gathered after the cell culture within a reaction vessel, and transplanting to other equipments which installed new porous membrane.
[Claim 11] The manufacture approach of the gene-fixation film characterized by to fix a gene in porous membrane after inoculating the host cell holding the vector which has a gene, cultivating this cell and performing magnification and purification of a gene in the reaction vessel of the gene-analysis equipment which consists of two or more reaction vessels formed by the pars basilaris ossis occipitalis formed of two or more porous membrane by which the laminating was carried out, and the septum formed in the upper part of porous membrane.

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